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Diallel analysis of tolerance to aluminium in alfalfa

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Summary

Acid soils having high levels of aluminium (Al) can drastically reduce yields in alfalfa and the most economically viable solution to the problem appears to be the development of Al-tolerant cultivars. To assist with the choice of a breeding method, a six-parent alfalfa diallel (crosses and reciprocals included but not parents) was evaluated in Al-toxic nutrient solution in terms of height (HT) and dry weight (DW). General combining ability was significant for both traits and constituted the majority of the genetic variation. Specific combining ability was significant only for HT and reciprocal effects were significant only for DW. Tolerance appeared to be at least partially dominant to sensitivity. Results indicate that a mass selection scheme, such as recurrent phenotypic selection, may be effective in increasing levels of tolerance in at least some alfalfa populations and that minor grains may also be achieved through exploiting non-additive genetic variation.

Introduction

An estimated 40% of arable soils and 70% of non-arable soils of the world are acidic (Osmond et al., 1984) and, in many of these soils, aluminium (Al) toxicity is the primary growth-limiting factor for plants (Foy, 1988; Long & Foy, 1970; Mallet et al., 1987). Excess soluble or exchangeable Al is especially undesirable in subsoils because it reduces rooting depth and branching and it predisposes plants to drought injury (Goldman et al., 1989a, 1989b; Kauffman & Gardner, 1978; Kennedy et al., 1987). In most soils, liming the plow layer does not neutralise phytotoxic Al in sub-surface layers and applying lime to subsoils is generally not economically feasible (Brooke et al., 1989; Foy, 1988; Kaufmann & Gardner, 1978; Long & Foy, 1970). In some instances, liming even the surface soil may not be feasible because soils must be kept acidic (below pH 5.4) for disease control, or because lime is unavailable or prohibitively expensive (Foy, 1988). In all of these situations, Al-tolerant plants offer an alternative or supplemental solution to the problem (Foy, 1983; Furlani, 1987; Jan & Pettersson, 1989; Little, 1988).

Bouton et al. (1986) determined that acid subsoils can reduce yields substantially in alfalfa; but that subsoil liming, gypsum application or, possibly, tolerant cultivars can be helpful in overcoming the problem. Rechcigl et al. (1986) concluded from nutrient solution studies that Al at a concentration of < 80 μmol was not detrimental to alfalfa seedling growth at pH 4.5. Reactions to Al stress in 23 alfalfa cultivars and checks representing a broad genetic base, including the entire range of dormancy types, were evaluated in soil (26.2% Al saturation (pH 4.8) versus 2.8% Al saturation (pH 5.7)) and in pH 4.5 nutrient solutions containing 0 or 111 μmol Al (Campbell et al., 1989). Genetic variation in response to toxic levels of Al was much more pronounced in nutrient solutions than in soil. Buss et al. (1975b) evaluated 18 alfalfa cultivars on unlimed (pH 4.4) and limed (pH 6.0) clay loam subsoil. They concluded that there was a narrow range of acid soil tolerance among these alfalfa cultivars, but that individual genotypes differed in their reaction to low and high pH. Bouton & Sumner (1983) noted that two populations selected for acid-soil tolerance produced significantly higher yields than the control when data were pooled over four soil pH levels, but that the selections offered no significant advantage in low pH soils. In these studies, acid-soil-tolerant selections were more responsive to phosphorus application than the controls over all pH levels. Devine et al. (1976) demonstrated that an alfalfa population (AT-3) which had undergone two cycles of selection for tolerance to Al in acid soil (pH 4.1 to 4.5) had significantly greater top and root vigor when grown in acid soil (pH 4.6) than did a population (AS-3) which had been subjected to two cycles of selection for sensitivity to Al in acid soil. They noted a strong correlation between top and root vigor and concluded that effective selection could be based on top-growth evaluation alone. The alfalfa synthetic B13-A14 was developed with four cycles of phenotypic recurrent selection under Al toxic conditions (Campbell et al., 1988). Screening for cycles 1 and 2 was in soil (pH 4.7–4.9) and subsequent screening was in nutrient solution (pH 4.5). This synthetic was slightly more vigorous in Al-toxic soil (pH 4.6) than eight other entries and was among the least impaired in the ability to take up nutrients under Al stress in nutrient solutions (pH 4.5; 111 μmol Al) compared to seven other entries. Campbell et al. (1988) concluded that the screening procedure used was much more effective in selecting for vigor than for Al tolerance.

The objective of the current study was to conduct a diallel analysis of tolerance to Al in alfalfa as a basis for developing more efficacious breeding procedures.

Materials and methods

Selection of parents

Two sensitive (S2 and S8), one moderately sensitive (MS1), two moderately tolerant (MT7 and MT10), and one tolerant (T1) parents were selected, based on their relative weights (fresh weight with Al stress/fresh weight without Al stress; Table 1) when evaluated (Baligar et al., 1992) in a replicated pot study in Porter soil (coarse-loamy, mixed mesic, Umbric Dystrochrept). To provide the differential stress, Al saturation levels were adjusted with dolomitic lime to 0.3% (pH 5.3) and 59% (pH 4.1). MS1, MT7, and MT10 trace to the population AT-4 (population AT-3 with an additional cycle of selection in acid subsoil for tolerance to Al), and S2, S8, and T1 trace to the cultivar Williamsburg. Coefficients of variation (Table 1) are typical of such evaluations and serve to emphasise the substantial genotype × environment interaction encountered when screening alfalfa for tolerance to Al in soil.

Development of diallel

The diallel conformed to Griffing's (1956) Method 3, Model I, where one set of F_1's and reciprocal

Table 1. Mean responses of six alfalfa clones to toxic levels of Al in a Porter soil

Clone	Relative wt. (wt. stressed/wt. unstressed) (%)	CV (%)	Fresh wt. unstressed (g)	CV (%)
S2	7.4	96.5	2.2	36.8
S8	5.5	118.9	1.3	22.2
MS1	23.8	48.8	1.4	14.2
MT7	64.1	128.5	2.1	74.8
MT10	50.3	64.8	1.8	77.1
T1	119.4	86.1	1.0	83.5

crosses are included, but not parents, and where replications are random and parents fixed. No emasculation was performed.

Evaluation of diallel

To minimise environmental variation, progenies were evaluated in aerated, modified Steinberg solution (Foy et al., 1967) in glass tanks with interior dimensions of 76.2 cm (l) × 30.5 cm (w) × 30.5 cm (d) and painted black outside to exclude light. Tanks were filled to within 6 cm of the top with solution; black plastic boards [70 (l) × 28 (w) × 0.5 cm (d)] with 26 rows of 2 mm holes (countersunk 0.25 cm) and 14 holes row^{-1} were suspended at the surface of the solution. Hole spacing was 2 cm between and within rows. Solutions were changed every 7 d and a pH of 4.5 was maintained by the addition of 1 N HCL or 1 N NAOH. One germinated seed with a radicle length of approximately 1 cm was placed in each hole. Two adjacent rows constituted an experimental unit. Numbers of seedlings per experimental unit ranged from 14 to 27 depending on seed availability. The check populations B13-A14 and AS-4 were included in the experiment. Experimental design was a randomised complete block with four replications and there were three tanks per replication. Plants were grown in the growth chamber at 27° C and an 8 h photoperiod (provided by 64 cool-white fluorescent tubes (F96T12) and eight 100 W clear incandescent bulbs; photosynthetic photon flux was 200 μmols s^{-1} m^{-2} measured at 0.86 m from the light source). Aluminium stress was maintained at 111 μmol for 7 d, then at 222 μmol for 21 d when heights (HTs) of individual plants were taken. Plants were dried at 70° C for 7 d in a forced-draft oven and weighed.

Analyses of data

Analyses of variance as described by Griffing (1956) were conducted on each response variable. Using Griffing's (1956) formulae, General Combining Ability (GCA), Specific Combining Ability (SCA), and reciprocal effects were estimated as were GCA and SCA variances for each parent.

Results and discussion

The range of mean HTs was 13.2 to 24.3 cm, and the range of mean dry weights (DWs) was 44.7 to

Table 2. Effect of Al toxicity on mean progeny height (cm) and dry weight (mg) in a six-parent alfalfa diallel. Within each cell, height is presented first and dry weight second

♀ / ♂	S2	S8	MS1	MT7	MT10	T1	Mean
S2	–	13.3	15.6	16.1	15.7	19.8	16.1
	–	44.7	64.9	72.8	68.5	92.1	68.6
S8	15.0	–	17.0	16.3	14.3	22.6	17.0
	64.9	–	70.4	71.2	46.4	121.7	74.9
MS1	13.8	13.6	–	16.8	15.9	17.9	15.6
	55.9	47.0	–	60.0	56.9	71.1	58.2
MT7	14.4	13.2	15.5	–	14.3	24.3	16.3
	52.0	51.8	53.5	–	48.3	109.1	62.9
MT10	16.5	15.3	15.0	15.3	–	18.1	16.0
	74.3	47.0	56.5	58.5	–	91.3	65.5
T1	18.3	19.6	18.9	20.9	16.9	–	18.9
	67.4	71.0	81.3	91.5	76.9	–	77.6
Mean	15.6	15.0	16.4	17.0	15.4	20.6	–
	62.9	52.3	65.3	70.8	59.4	97.1	–

121.7 mg (Table 2). Mean HTs for AS-4 and B13-A14 were 13.3 and 22.9 cm, and mean DWs were 55.9 and 113.1 mg, indicating that the Al stress imposed was sufficient to induce differential responses. General combining ability was significant for both response variables and constituted the majority of the genetic variation (Table 3). Specific combining ability was significant only for HT, whereas reciprocal effects were significant only for DW (Table 3). Despite these differences, high correlations between HT and DW ($r = 0.91^{**}$, df = 120) indicate that these variables were equivalent indicators of reaction to Al toxicity in this study. Based on contrasts, the only significant (0.01 level) reciprocal effect was S8 × T1 (121.7 mg) versus T1 × S8 (71.0 mg). If maternal effects were involved in this case, the larger mean should have been derived from the cross T1 × S8; because it was not, other causal factors such as differential maternal nutrition may have been involved. Regardless of the cause of the significant reciprocal effects, it appears that reciprocal effects as well as autogamy (Dudley, 1963) were minor contributors to the total genetic variation. Relative weights of the parents in soil (Table 1) were significantly correlated with mean responses of parents in hybrid combination ($r = 0.88^{*}$ and 0.85^{*} for HT and DW, respectively). Although responses to Al are probably confounded with variations in vigor, these strong correlations indicate that much of the genetic variation observed was due to the effects of Al.

T1 was tolerant, had the greatest GCA, and was conspicuously different from the other parents in hybrid combination (Table 2 and 4). T1 was also the least vigorous of the parents (Table 1) indicating that tolerance in this clone was not related to vigor. MT10 demonstrated more sensitivity than would have been predicted from its response in soil. This response represents not only the effects of minor non-additive genetic variation but also, perhaps, the effects that substantial genotype × environment interaction can have on the precision with which responses to Al can be estimated in soil and the fact that responses in soil and nutrient solution are not always strongly correlated (Campbell et al., 1988).

Progenies from susceptible × susceptible crosses performed as expected (Table 2). Progenies from crosses involving T1 were relatively tolerant indicating that tolerance is at least partially dominant to sensitivity. However, progenies from crosses involving MT7 or MT10 were generally more sensitive than expected based on the performance of these clones in soil. Of the specific crosses, S2 × MT10, S8 × T1, and MT7 × T1 contributed significantly to the total SCA variation (Table 5) and it is interesting to note that two of these crosses involve T1. It appears that the lack of precision in estimating responses to Al-toxic soil (Table 1) had its greatest impact on the selection of marginally tolerant types. MT7 and MT10 were probably more sensitive than the soil test indicated and perhaps more likely to perform differentially in soil versus nutrient solution than the highly sensitive or tolerant selections.

Parents in this diallel were not random samples from populations in random mating equilibrium, thus any genetic interpretation of the analyses must be done with caution. However, the size of the GCA mean square indicates that additive gene action may be the most important source of genetic varia-

Table 3. Mean squares from an analysis of variance of a six-parent alfalfa diallel evaluated in Al-toxic nutrient solution

Source	df	Height (cm)	Dry weight (mg)
GCA	5	118.1**	4703.6**
SCA	9	15.3*	624.8
Reciprocal effects	15	8.2	776.8**
Error	87	7.6	328.7
CV (%)		16.5	26.7

*, ** Significant at the 0.05 and 0.01 levels, respectively.

Table 4. General Combining Ability effects ($\hat{g}_i$) from a six-parent alfalfa diallel evaluated in Al-toxic nutrient solution

Parent	Height (cm)	Dry weight (mg)
S2	− 0.97	− 2.7
S8	− 0.80	− 5.2
MS1	− 0.80	− 7.5
MT7	0.03	− 1.3
MT10	− 1.13	− 6.6
T1	3.70	23.3
LSD (0.05)	2.73	18.0

Table 5. Specific Combining Ability effects ($\hat{s}_{ij}$) from a six-parent alfalfa diallel evaluated in Al-toxic nutrient solution

Cross	Height (cm)	Dry weight (mg)
S2 × S8	0.69	– 4.95
S2 × MS1	– 0.10	2.99
S2 × MT7	– 0.45	– 1.40
S2 × MT10	1.63	13.09
S2 × T1	– 0.43	– 9.68
S8 × MS1	0.28	3.95
S8 × MT7	– 1.12	0.36
S8 × MT10	0.12	– 8.95
S8 × T1	1.41	9.64
MS1 × MT7	0.25	– 2.05
MS1 × MT10	0.81	3.39
MS1 × T1	– 1.25	– 8.23
MT7 × MT10	– 0.74	– 6.30
MT7 × T1	2.06	9.49
MT10 × T1	– 1.18	– 1.18
LSD (0.05)	3.86	25.46

tion for reaction to toxic levels of Al and that a mass selection scheme such as recurrent phenotypic selection would be effective in increasing levels of tolerance in at least some alfalfa populations. Based on theoretical considerations of autotetraploid breeding by Rowe & Hill (1985), mass selection can be more effective in population improvement than many schemes involving progeny testing unless the heritability of a trait is very low. The large variances (Table 6) associated with the only stable tolerant parent, T1, indicate that it would not be completely suitable for inclusion in a synthetic but would be best used in hybrid combination. However, the utility of such clones would ultimately be contingent upon the heritability of tolerance and upon the complexity of inheritance. Certainly, combining ability analysis of a large number of potential contributors to a synthetic could be prohibitively costly and time consuming.

The use of recurrent phenotypic selection, as employed by Campbell et al. (1988), was only marginally effective. They concluded that progress may have been impeded by substantial genotype × environmental variation and that a more effective approach would entail propagating each selection clonally and re-evaluating it in replicated studies before final selections were made. Results of the current study also indicate that there could be considerable risk associated with selecting intermediate types as part of a breeding program. The authors also advanced the possibility of employing in vitro selection, partially to reduce the effects of environmental variation. In a later study, Parrott & Bouton (1990) noted excellent correlations between reactions of individual clones to Al toxocity in vitro and in soil. They concluded that a strategy based on in vitro callus culture could be used effectively to assist in the selection of acid or Al-tolerant genotypes for use as parents in a breeding program.

Although SCA was a minor source of variation, attempting to capitalise on non-additive genetic variation by choosing particular crosses that yielded unusually tolerant progenies may also be effective. Unfortunately, producing seed on a commercial basis from small numbers of parents is often economically prohibitive, although advances in artificial seed technology (Stuart et al., 1987) offer some

Table 6. Estimates of General and Specific Combining Ability variances ($\hat{\sigma}g_i^2$ and $\hat{\sigma}s_i^2$, respectively) from a six-parent alfalfa diallel evaluated in Al-toxic nutrient solution

Parent	Height (cm)		Dry weight (mg)	
	$\hat{\sigma}g_i^2$	$\hat{\sigma}s_i^2$	$\hat{\sigma}g_i^2$	$\hat{\sigma}s_i^2$
S2	0.55	– 0.61	– 10.20	13.43
S8	0.23	– 0.54	9.95	– 8.34
MS1	0.25	– 0.81	38.39	– 34.68
MT7	– 0.40	0.18	– 15.50	– 27.64
MT10	0.88	0.39	26.30	14.34
T1	13.14	1.42	524.33	24.74

hope that the cost of establishment could be reduced.

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